IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit: 1646
	)	
David WALLACH et al	)	Examiner: G. Draper
	)	
Appln. No.: 08/474,691	)	Washington, D.C.
	)	
Filed: June 7, 1995	)	
	)	
For: TUMOR NECROSIS FACTOR INHIBITORY PROTEIN AND ITS PURIFICATION	)	Atty.Docket: WALLACH=1B

DECLARATION UNDER 37 C. F. R. §1.132

We, Hartmut Engelmann and David Wallach, hereby declare and state as follows:

Each of us is a co-inventor of the above-identified application and a co-author of Engelmann et al, "A Tumor Necrosis Factor-Binding Protein Purified to Homogeneity from Human Urine Protects Cells from Tumor Necrosis Factor Toxicity", J. Biol. Chem. 264:11974-11980 (1989), hereinafter the "Engelmann publication".

The curriculum vitae of Hartmut Engelmann is attached hereto as Exhibit A.

The curriculum vitae of David Wallach is attached hereto as Exhibit B.

The above-identified patent application teaches those skilled in the art how to obtain a substantially purified protein, obtainable from human urine, capable of interacting with TNF so as to inhibit the binding of TNF to cells and to inhibit the cytotoxic effect of TNF, which protein contains the following amino acid sequence: Asp-Ser-Val-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-Hsp-Pro-Gln-X-Asn-Ser, wherein X is an unidentified amino acid

residue. On information and belief, this invention is claimed in claim 28, as will be amended on even date with the filing of the present declaration.

All of the experiments described in the above-identified application and in the Engelmann publication were performed directly by Hartmut Engelmann and/or under the supervision of David Wallach, and we hereby declare that that all of the results described in the above-identified application and in the Engelmann publication are true and accurate to the best of our knowledge and belief.

In the course of the experimentation, the specific activity of TNF-binding protein was measured. Although the results were not set forth in the patent application, they appear in Table II in the second column of page 11978 of the Engelmann publication. As with the other experimental results in the Engelmann publication, the specific activity measurements as set forth in Table II were conducted by Hartmut Engelmann and/or or under the supervision of David Wallach, and the results are true and correct to the best of our knowledge and belief.

The method described at pages 15-19 of the specification of the above-identified application for the purification of the TNF inhibitory protein permits obtaining a substantially purified protein. This substantially purified protein has a specific activity of about 600,000 units/mg, as stated in Table II of the Engelmann publication.

The process described in the above-identified application involves, first, obtaining crude urinary proteins by

filtering urine on a Pellicon membrane with a pore size of 0.5 μm, and then concentrating by ultrafiltration using a Pellicon membrane with a molecular weight cut-off of 10 kDa. This is described in paragraph 3.1, bridging pages 16 and 17 of the above-identified application. It can be seen that these are the same steps as are described in the second column of page 11975 of the Engelmann publication, under the section "Purification of the TNF-binding Protein", in the paragraph entitled "Concentration of the Crude Urinary Proteins (CUP)."

The first line of Table II of the Engelmann publication, page 11978, states that the specific activity of the crude urinary protein, which was the starting material for the subsequent purification steps, as obtained by the above-described urinary filtration and concentration, was 13 units/mg.

In paragraph 3.2 on page 17 of the above-identified application, the carboxy methyl (CM) Sepharose chromatography purification step is described. It can be seen from the experimental conditions that the experiment being described is the same as that described in the paragraph entitled "Chromatography on CM-Sepharose" at page 11975 of the Engelmann publication. Table II of the Engelmann publication shows that the specific activity of the product of the CM-Sepharose step was 610 units/mg.

Paragraph 3.3, bridging pages 17 and 18 of the above-identified application, describes the step of cation-exchange Mono S HR 5/5 chromatography. The bound proteins were eluted with the linear NaCl gradient in buffer A, run for 40 minutes at a flow

rate of 0.5 ml/minute. It can be seen from the paragraph entitled "Cation Exchange HPLC", on page 11975 of the Engelmann publication, that the experimental conditions described in the Engelmann publication are identical to those described in the above-identified application. The specific activity after this step, as shown in Table II of the Engelmann publication, was 1,120 units/mg.

In paragraph 3.4 at page 18 of the above-identified application, the step of anion exchange Mono Q HR 5/5 chromatography was described. Again, it can be seen by comparison with the paragraph entitled "Anion Exchange HPLC", bridging pages 11975 and 11976 of the Engelmann publication, that the conditions described in the above-identified application and in the Engelmann publication are identical. The specific activity of the active fraction from the Mono Q column, as shown in Table II of the Engelmann publication, was 29,100 units/mg.

Finally, paragraph 3.5 on page 19 of the above-identified application, describes the step of reversed-phase HPLC on an RP 300 column. By comparison of this paragraph with the paragraph entitled "Reversed Phase HPLC", at page 11976 of the Engelmann publication, it can be seen that all of the materials and gradient conditions were identical in both descriptions. As stated in Table II of the Engelmann publication, the result of this HPLC step on an RP 300 column included active fractions with a specific activity of 600,000 units/mg.

Thus, the specific activity data in Table II in the Engelmann publication describes the specific activity inherently

obtained by the purification steps described in the above-identified application, as both methods are the same. Both descriptions describe the same experiments performed by Hartmut Engelmann and/or supervised by David Wallach. Therefore, the substantially purified protein obtained by the methods described in the above-identified application have a specific activity of about 600,000 units/mg.

It is understood that the prior art, cited by the examiner, is Peetre et al, "A Tumor Necrosis Factor Binding Protein Is Present in Human Biological Fluids", Eur. J. Haematol. 41:414-419 (1988) (hereinafter "the Peetre publication") and Seckinger et al, "A Human Inhibitor of Tumor Necrosis Factor α", J. Exp. Med. 167:1511-1516 (1988) hereinafter "the Seckinger publication"). A review of the methods of purification described in the Peetre and Seckinger publications, however, show that only a relatively crude product is obtained. Indeed, in Table 1, at page 417, of the Peetre publication, the maximum specific activity is described as 280 units/mg. There are over three orders of magnitude difference in the specific activity of the substantially purified protein which can be obtained by the process described in the above-identified application, as compared to the relatively crude urinary protein of the Peetre publication. The process of the Seckinger publication cannot obtain purification substantially better than that described by the Peetre publication. Thus, neither reference disclose substantially purified protein. As discussed above, the substantially purified protein obtainable by the process of the above-identified application has a specific

activity of 600,000 units/mg. Such substantially purified protein was not described in the prior art as of the publication dates of the Peetre and Seckinger publications.

Furthermore, in our opinion, those of ordinary skill in the art at the time that the Peetre and Seckinger publications were published would not have found it obvious how to obtain the substantially purified protein of the present invention starting with the crude preparations of Peetre and Seckinger.

The undersigned declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2/2/99
Date

Hartmut Engelmann

2/2/99
Date

David Wallach

[illegible]

Curriculum Vitae

Personal Data

Name	Hartmut Engelmann, M. D., Ph. D.
Date of Birth	26.10.1958
Place of Birth	Prien/Chiemsee, Deutschland

Education

Elementary School	*1965 - 1969,* Implerschule in Munich
High School	1969 -1978, Klenze Gymnasium in Munich, Graduation (Abitur) in June 1978
University	*Nov. 1978 - April 1986:* Medical School at the Ludwig Maximilians University in Munich
	(*Nov. 1979 - Oct. 1980:* Interruption of Medical School for Civil Service duties see below)
Clinical Rotations	*April 1985 - August 1985,* Internal Medicine: Municipality Hospital Munich Harlaching
	August 1985 - Nov. 1985, Surgery: Municipality Hospital Landshut
	Nov. 1985 - March 1986, Pediatrics: Pediatrics Department of the University Hospital Munich
	Graduation from Medical School (Staatsexamen) April 1986
	Approbation as M. D. June 1986

Civil Service Duties

Nov. 1979 - Oct. 1980: Intensive Care Unit for Newborn at the University Hospital Grosshadern, Munich

Scientific Work

Sept. 1986 - Aug. 1991: Scientific assistant in the laboratory of Prof. David Wallach at the Weizmann Institute of Science, Dept. of Molecular Genetics and Virology (Director Profs. M. Revel and Yoram Gruner)

During this time

Dissertation work for M. D. (Dr. med.) Subject: *"Molcular Analysis of the Type I (p55) Tumor Necosis Factor Receptor Signalling Mechanism"* under the supervision of Prof. Dr. Wallach (Weizmann Institute of Science and Prof. Dr. Riethmüller (Institute of Immunology, Munich University; summa cum laude)

Dissertation work for "Doctor of Philosophy" (Subject: *"Molecular Mechanisms Controlling the Action of Tumor Necrosis Factor (TNF)"*; under the supervision of Prof. David Wallach and Prof. Michel Revel at the Weizmann Institute of Science)

since Sept 1991 Senior Scientist at the Institute of Immunology, Munich University, and head of an independent research laboratory
Major research interest *"Regulation of programmed cell death by recpetors of the TNF/ NGF Receptor-Family (TNF Receptor, CD40 and Fas/Apol)"*

Grants

SFB217 (Programmed Project) Support the 3rd 3 years period since 1991
Subjects
"Molecular Mechanism of Monocyte Activation: Regulation and Function of the CD14 Membrane-Antigen"
und
"CD40 Signaltransduction in Non-Hemtopoetic Cells" .

DFG (in the framework of the Gerhard Hess Program): since 1993
Subject: "Regulation of Programmed Cell Death (Apoptosis): Molecular Analysis of Anti-Apoptotic Signals Mediated by CD40"

Stipends und Prices

Jan. 1987 - Dec. 1989 Minerva Fellow
1993 Gerhard Hess Price of the DFG

EXHIBIT B

DAVID WALLACH

Date & Place of Birth:	23 January, 1946, Haifa, Israel
Marital Status:	Married, 2 children
Nationality:	Israeli
Military Service:	Israel Defense Forces (1964-1966)

EDUCATION:

1966-1968	B.Sc.	The Hebrew University of Jerusalem Department of Biological Chemistry Studies in Biochemistry and Physiology
1968-1969	M.Sc. (with distinction)	The Hebrew University of Jerusalem Department of Biological Chemistry Thesis: "Photosynthetic Activities during Chloroplast Membrane Biogenesis in Chlamydomonas reinhardi y-1", under the guidance of Professor I. Ohad
1970-1974	Ph.D.	The Hebrew University of Jerusalem Department of Biological Chemistry Thesis: "Components of the membrane and components of the content of the secretory granules in the rat parotid gland", under the guidance of Professor M. Schramm

RESEARCH EXPERIENCE:

1970-1973	Research Assistant	The Hebrew University of Jerusalem Jerusalem, Israel
1973-1974	Instructor	The Hebrew University of Jerusalem Jerusalem, Israel
1974-1977	Visiting Fellow	Laboratory of Molecular Biology National Cancer Institute National Institutes of Health Bethesda, Maryland 20014, USA

1977-1978	Fellow	Department of Virology The Weizmann Institute of Science Rehovot, Israel
1979-1983	Senior Scientist	Department of Virology The Weizmann Institute of Science Rehovot, Israel
1983-1984	Associate Professor	Department of Virology The Weizmann Institute of Science Rehovot, Israel
1985-1992	Visiting Scientist	Department of Virology The Weizmann Institute of Science Rehovot, Israel
1992-1995	Associate Professor	Department of Membrane Research and Biophysics The Weizmann Institute of Science Rehovot, Israel
1995-	Full Professor	Department of Biological Chemistry The Weizmann Institute of Science Rehovot, Israel